Characterization and evaluation of a fungal growth medium composed pollens powder of cattail (*Typha domingensis Pers.*)

Caracterización y evaluación de un medio de crecimiento fúngico compuesto por pólenes en polvo de espadaña *(Typha domingensis Pers.)*

Autor:

Salah M. Al-Bader [1]

ARTÍCULO DE INVESTIGACIÓN CIENTÍFICA Y TECNOLÓGICA

How to cite this paper:
Al-Bader S M., Characterization and evaluation of a fungal growth medium composed pollens powder of cattail (*Typha domingensis Pers.*). Innovaciencia Facultad de Ciencias Exactas, Naturales y Agropecuarias. 2018; 6(1) S1: 1-7.

Reception date:
Received: 15 August 2018
Accepted: 25 November 2018
Published Online: 28 December 2018

DOI:
http://dx.doi.org/10.15649/2346075X.480

Keywords:
Typha, Cattail, pollen, medium, fungi.

ABSTRACT

Introduction: A great progressing occurs in microbiology after culture media have been used in culturing, isolation, identification, and reservation of microorganisms. According to their components, culture media were divided into natural, synthetic, and semisynthetic, the natural medium contain almost all the nutritional requirements but in non-defined quantities. In the current work, the validity of *Typha domingensis* pollens as a new substrate for fungal culture medium was tested. Several physical and chemical characteristics of medium were detected. The growth of nine fungal isolates were evaluated on semisolid and broth media. **Materials and Methods**: Ten gram of starchy powder free from spike tissue + 15gm agar were used for one liter of Typha pollens agar (TPA). The growth of local isolates of *Candida sp., Rhodotorula sp., Aspergillus niger, Ulocladium sp., Cladosporium sp., Tri[illegible]ma sp., Penicillium sp.*, *Chrysosporium sp.*, and *Microsporum sp.* were tested on (TPA), and (PCA). Biomass of filamentous isolates was estimated by TP broth and PC broth. ANOVA test at level 0.05 was followed to clarify the significant increasing of biomass. To qualify the nutritional value of Typha pollens, the percentage of total nitrogen , carbon , phosphorus , and potassium were estimated . pH of TPA were detected as well as its color, transparency , and gelatin texture were practically evaluated . **Results and Discussion** : The chemical composition analysis showed that pollens powder contain C=58%, N=2.16%, P= 0.19% and K=3.36%. The pH was 6.72 at room temperature . Typha pollens agar support growth of all tested fungal isolates. The semisolid TPA had a typical characteristic for culturing and diagnosis of fungi including a suitable gelatin texture and a pale transparent yellow color . All tested fungi except *Penicillium sp.* showed higher growth on TPA , on the other side the fungal biomass increased significantly by TP broth in comparison with PC broth (ANOVA test at 0.05 level). **Conclusions** : Pollens of cattail (*T. domingensis*) provide a suitable environment , and nutritive requirements for fungi .It can be used easily and successfully as essential substrate for preparing culture medium for primary isolation of fungi. A farther studies were needed to gain a view about using *T.domingensis* pollens to isolate pathogenic fungi and bacteria.

[1] Knowledge University, Erbil-Kurdistan region-Iraq, e-mail: Salah.saleem@knowledge.edu.krd.

INTRODUCCTION

Cultures media: They play a critical role in developing microbiology where they are used for isolation, culturing, and preservation of microbes. A microbiological culture medium is a nutritive composition that provide an appropriate environmental condition for microorganisms to survive far from their natural habitats. They are fundamental requirement in all branches of microbiology such as medical microbiology, industrial microbiology, and microbial genetic [(1)]. Cultures media were divided according to physical base into solid, semisolid, and liquid [(2)], while they were divided on basis of composition into natural, and semisynthetic [(3)]. The natural culture media are easily prepared but they have undefined ingredients. Culture medium should provide at least the minimum level of best growth requirements. Cattail (*Typha domingensis*): It is a perennial, herbaceous, welt land, and a cosmopolitan plant. Cattail is one of the predominate aquatic and semiaquatic plants in marshes and swamps of central and south Iraq. The vegetative parts are used in several traditional industries, and folk remediation. Cooked or fresh rhizomes are consume, they are tasty, nutritive and a good source of energy [(4)]. The roots are useful in treating burns and intestinal disorders (DOE) [(5)]. Pollens had been used in the treatment of bleeding, and a numerous disorders of gastrointestinal and urinary system[(6)]. In south Iraq, beside the folk medical uses, pollens are used to prepare a native sweet called "khurrait". Culture media: They are a fundamental requirement in all microbiological fields. Generally, growth medium should provide a sufficient amounts of water, and appropriate pH beside the macro-molecules and micro-molecules which are essential for growth of microorganisms [(7)]. Growth medium for fungi commonly contains a plant component as a rich source of carbon in addition to other nutritional requirements [(8)]. Bean, hay, potato, carrot, corn, tomato juice, wood extract etc were used as the main part of fungal nutrition media [(9,10)]. Looking for a new growth media was a goal of several works for both macro and micro fungi [(11, 12, 13)]. The current study was conducted to evaluate pollens of *Typha domingensis* as natural, nutritive, locally available, and cheap component to prepare solid and broth fungal growth media.

MATERIALS AND METHOD

The culture medium: In April 18, 2018, 500 gm of dry spike powder were brought from Basrah market-south of Iraq (figure-1 left). A metal sieve with fine pores was used to separate pollens from spike fine debris (hairs).

Figure 1. The starchy pollens of *T. domingesis* (left) , suspention of pollens powder with agar before autoclaving(right).

Pollen powder was kept in glass container at 4°C until using. To prepare the medium (I suggest "Typha pollens agar- TPA" to use here), 10gm of pollens and 15gm agar were mixed and was added to one liter of distilled water. The suspension (figer-1 right) was carefully mixed by hot plat magnetic stirrer (5 min/60°C). After autoclaving (15 min at 121°C and 1Kg/cm^2), the antibiotic chloramphenicol was added to inhibit bacterial growth. Potato dextrose agar (PDA), Sabouraud's dextrose agar (SDA), and potato carrot agar (PCA) were prepared. Broth media of Typha pollens (TPB) and potato carrot (PCB) were prepared also. The acidity of TPA was measure by pH meter model "BP 3001-TRANS Instrument" at room temperature after autoclaving and before solidifying. The tested fungi: They were isolated from several habitats during April and May 2018 (Table-1).

Table-1. Fungi that were tested on potato carrot agar and Typha pollens agar, and their sources

	Fungi	Source
1	*Aspergillus niger*	Air
2	*Candida sp.*	Mouth swab
3	*Chrysosporium sp .*	Feathers
4	*Cladosporium sp .*	Air
5	*Microsporum sp .*	Wool
6	*Penicillium sp .*	Air
7	*Rhodotorula sp.*	Skin swab
8	*Trichoderma sp.*	Corn seeds
9	*Ulocladium sp.*	Air

All isolates were recognized morphologically based on [14, 15, 16, 17] . Primary isolation had been done on TPA , then a pure cultures were prepared. To illustrate the morphological characteristics of fungal growth , a PCA and TPA plates were inoculated centrally by fungal isolates. A duplicate were conducted and were incubated at 30°C. The plates were carefully checked for ten days, and the morphological characteristics of fungal growth, colony expanding and colony feature, density and sporulation had been carefully recorded (daily for 10 days). In a 100ml sterile conical flasks, 50ml of potato carrot broth (PCB) and Typha pollens broth (TPB) were inoculated by a disc of fungal pure cultures (5 mm in diameter). They were incubated in room temperature for 10 days . The mycelia were collected by fine metallic sieve and was washed several times by distilled water, then were dried at 70°C on whatman no.1 filter paper [11] . The weight of fungal biomass was calculated as a mean of duplicates by following equation: Weight of fungal biomass= [weight of filter paper (gm) + weight of mycilium (gm)] - weight of filter paper (gm) .An electrical balance (KERN-$_{PFB}$ 200-3) was used to weight the dry fungal biomass. The tests were duplicated by using 6 month old pollens powder to check the effect of storage on powder quality.

RESULTS

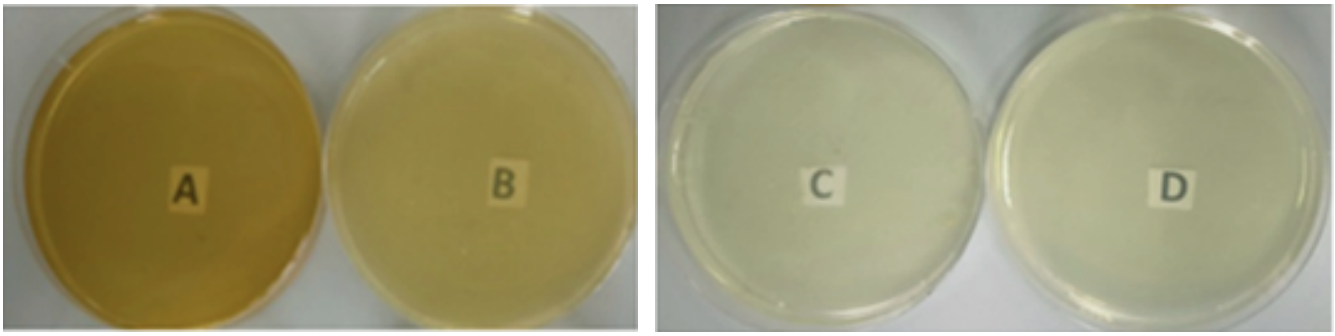
General characteristics of TPA/ The results showed that Typha pollen agar was easily prepared. The starchy pollens of cattail could be mixed with agar directly with no need for prefabrication processes . Pollens powder (figure- 1 left), unlike the other natural substrates (potato, carrot, banana peels and sugarcane bagasse... etc.) doesn't require washing, drying, cutting and grinding [18, 19, 20].

Figure 1. Powder of Typha pollen (left) and Typh pollen medium (right).

Furthermore the starchy pollens powder gave a homogenized mixture before and after autoclaving (figure-1 right). Chemical characteristics/ pH of pollens suspension was (6.6) at room temperature, it was within the optimal pH rang for fungi (between 5-7), and was slightly less than that had been recorded by Almosa and Abu Megdad [21, 22]. Estimation of macro elements showed that pollens contain C=%58, N=2.16%, P=0.19%, and S=3.36%. Generally fungi need a high source of carbon and nitrogen in growth media [3]. The percentage for these elements as well as the other nutrients are usually variable in natural growth media.

Optical characteristics of TPA/ Typha pollens agar seem to be pale yellow, clear, highly transparency and without debris or precipitations in the bottom of Petri dishes (figure-2). It was more clear than PDA and SDA but not PCA. The unclouded appearance of PCA related to using a double layers gauze to filtrate infusions of boiled potato and carrot.

Figure-2. Transparency of the four culture media (ligands under the plates).A=SDA; B=PDA; C=TPA; D=PCA.



Fungal growth / All the nine tested fungi were developed on both natural media TPA and PCA ,Eight of them (88%) seemed to produce higher mycelial and yeasts development, as well as sporulation density on TPA than on PCA (plate-1).

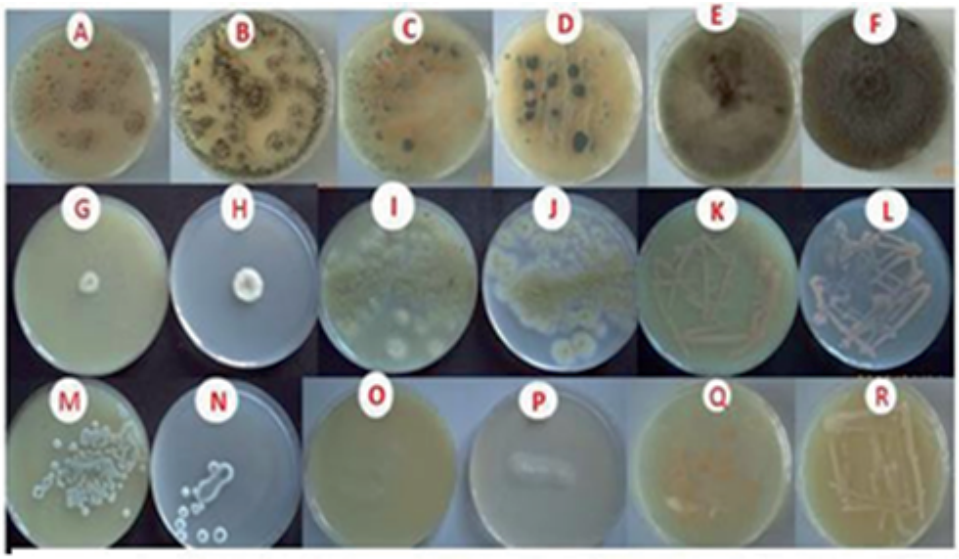


Plate-1 Growth of fungal isolates on TPA(B,D,F,H,J,L,N,P,R) and PCA(A,C.E,G,I,K,M,O,Q)

(A,B=*Aspergillus niger*) (C,D=*Cladosporium*) (E,F=*Ulocladium*) (G,H=*Microsporum*)
(I,J=*Trichoderma*) (K,L=*Rhodotorula*) (M,N=*Penicillium*) (O,P=*Chrysosporium*) (Q,R=*Candida*)

Penicillium was the only isolate which developed on PCA more than on TPA. The variations of fungal growth mainly refer to the chemical composition of substrate beside the diversity of enzymatic activity of the fungal isolates . Several studies [22,23,24,25] confirmed that carbon (C) and nitrogen (N), beside the C/N ratio are the most nutritional effective factor . The macro with micro elements as well as the type and the quantities of vitamins and the co-factors have a potential effects on fungal growth and should not be neglected. The significance of pH should not excluded hence it is a key factor for enzymatic activity Growth characteristics on semisolid TPA and the dry weight (gm) of filamentous fungi (table-2) showed that only *Penicillium* sp. created less growth and weight in pollens substrate than in potato carrot.

Table-2: Growth of fungal isolates on PCA and TPA media, and the dry weight (gm) of filamentous isolates on PCB and TPB.

	Fungi	Growth	PCB	TPB
1	*Aspergillus niger*	+	0.05	0.081
2	*Candida* sp.	+	Not tested	Not tested
3	*Chrysosporium* sp.	+	0.05	0.780
4	*Cladosporium* sp.	+	0.05	0.049
5	*Microsporum* sp.	+	0.03	0.066
6	*Penicillium* sp.	+	0.054	0.062
7	*Rhodotorula* sp.	+	Not tested	Not tested
8	*Trichoderma* sp.	+	0.05	0.058
9	*Ulocladium* sp.	+	0.047	0.050

ANOVA test at level 0.05 showed a significant increasing of total mean of fungal dry weight in Tupha pollen broth in comparison with potato carrot broth. (Appendix-1) The stored pollens for 6 months gave the same results and characteristics as fresh powder when was used to prepare the semi-solid medium. It is worth mentioning that stored pollen powder did not showed changes in its color and smell. The collectors of pollens from the plants in southern marshes of Iraq keep the spikes on a homemade plant mats for drying under direct sun light and not less than 40°C,such factors minimize microbial contamination and extending the validity of pollen powder.

CONCLUSION

Based on the finding of the study, Typha pollens agar and Typha pollens broth are a suitable medium for fungal growth. The semi-solid medium can be used successfully for primary fungal isolation, while the liquid medium no doubt need farther tests to be applicable in fungal physiology and biotechnology fields. Further studies should be done to explain the validity of TPA to isolate human pathogens, phytopathogenic, and toxogenic fungi as well as bacteria.

REFERENCES

1. Bridson E Y and Brecker A. Chapter III Design and Formulation of Microbial Culture Media. Methods in Microbiology, 1970; 3, part A: 229-295.
2. Sndle T. Assessment of culture media in pharmaceutical microbiology. American pharmaceutical review. 2014; 17(4).
3. Srijoni B, Chandra B, Nupur O, Nabajit. D, Jagaree D, Mrinmoy P, and Sukant K. Evolution of bacterial and fungal growth media. Bioinformation 2015; 11(4):182-184.
4. Tumer N J. Food plant of interior first peoples (Royal BC museum handbook). (2017). Published by university of British Colombia press, 1997.
5. Department of ecology (DEO) State of Washington. Native fresh water plants: cattail, a common and useful native plant. Retrieved. 2008.
6. Gupta A, Mishra AK, Bansal P, Kumar S, Sannd R, Gupta V, et al. Antileprotic Potential of Ethnomedicinal Herbs: A Review. Drug Invent Today. 2010; 2:191–3.
7. Harvey R.A, P.C. Champe, B.D. Fisher; Lippincott's. Illustrated Reviews: Microbiology, 2nd edition. Lippincott Williams & Wilkins: Philadelphia, 2007.
8. Omamor I B, A.O. Asemota, C.R. Eke, E.I. Eziashi. Fungal contaminants of the oil palm tissue culture in Nigerian institute for oil palm research (NIFOR). African Journal of Agricultural Research 2007; 2(10), pp.534-537.
9. Zakia A Osman, Sulieman M Elsanousi and Elisddig A Elmuafa Elsheik. Plant material as probable growth promoters for certain fungi. Asian journal of plant science and research. 2013; 3(1):87-93.
10. Saleem S. and Ali TA. Secreening of various plant extracts for their sutability to be used as growth promoting substances in the preparation of culture media for fungi. In the 117th general meeting of the American Society for Microbiology. 2014; Abs no. Q-416.
11. Senthamizhlselvan P, J. Alice R.P.Sujeetha and C. Jeyalakshmi. Growth, sporulation and biomass production of native entomopathogenic fungal isolates on a suitable medium. Journal of Biopesticides, 2010; 3(2): 466 – 469.
12. Abdullah S K and Al-Bader S M.Production of *Pleurotus sajor-caju* on date palm trees. Iraqi journal of Biology 2002; 2(1), 64-69.
13. Mekala U, Sevvel P, Nirmala R, and Sutharshiny S. Formulation of alternative culture media for bacterial and fungal growth. Der Pharmacia Letter, 2016; 8 (1):431-436.
14. Domsch KH, Gams W, Anderson TH. Compendium of soil fungi. IHW, Verlag, Eching, revised by Gams. 2007: 672.
15. De Hoog GS, Guarro J, Gene J, Figueras MJ. Atlas of Clinical Fungi Baarn: Centraalbureau voor Schimmel cultures 2001; 39: 71.
16. Seifert K, Morgan-Jones G, Gams W, Kindrik B. The genera of Hyphomycetes . CBS-KNAW. Fungal biodiversity center. Utrecht. The Netherlands. 2011: 997.
17. Al-Bader S. M.. A study of microfungal community in Mousal foest soil. Ph. D. thesis. Basrah University. 1996. Basrah Iraq.

18. Sidana A, and Farooq U.Sugarcane bagasse a potential medium for fungal culture. Chines journal of biology. 2014; article ID 840505, 5 pages.
19. Kirdo A J,Tupaki-Sreepruna A, and Yunaraj M. Banana peel culture as an indigenous media for easy identification of late sporulation human fungal pathogens. Indian journal of medical microbiology, 2016; 34(4):457-461.
20. Zhao,H. , Huang,L., Xiao, C. L., Liu, J., Wei, J. and Gao, X.. Influence of culture media and environmental factors on mycelial growth and Conidial Production of *Diplocarpon mali*. The Society for Applied Microbiology, litters in Applied Microbiology 2010; 50:639-644.
21. Almosa A A and Abu-Megdad N. Preparation of new and cheap culture growth medium from male inflorescences of *Typh domingensis*. Al-Mustansiyah journal of science, 2013; 24: 13-24.
22. Gao L, Sun M H, Liu X Z, Che Y S. Effects of carbon concentration and carbon to nitrogen ratio on the growth and sporulation of several biocontrol fungi. Mycol Res. 2007; 111 (Pt 1):87-92. Epub 2006 Dec 8.
23. Wu J Z, P. C. K. Cheung, K. H. Wong, and N. L. Huang, "Studies on submerged fermentation of *Pleurotus tuber-regium* (Fr.) Singer. Part 2: effect of carbon-to-nitrogen ratio of the culture medium on the content and composition of the mycelial dietary fibre," Food Chemistry, 2004; 85, (1):101–105.
24. Zhen Wang Z, Chun Yan Wang , Yun Bo Wang , Jian Jie Xue , Zheng Li , Jing Jie Li , Li Guan Gu and Chang Keun Sung. Effect of different pH values on growth and sporulation of *Estye vermicola* .African journal of microbiology research. 2013; 7(25) pp.3217-3221.
25. Al-Jubori M H and Hasan A M.Determination of Optimal Temperature and pH for Radial Growth of Some Dermatophytes Species Isolated from Leukemia Patients. Iraqi journal of science. 2015; 56(1A): 95-99.